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Reporting Summary

Nature Research wishes to improve the reproducibility of the work that we publish. This form provides structure for consistency and transparency in reporting. For further information on Nature Research policies, see Authors & Referees and the Editorial Policy Checklist.

Statistics

For all statistical analyses, confirm that the following items are present in the figure legend, table legend, main text, or Methods section.

n/a	Confirmed	
☐	☒	The exact sample size (*n*) for each experimental group/condition, given as a discrete number and unit of measurement
☐	☒	A statement on whether measurements were taken from distinct samples or whether the same sample was measured repeatedly
☐	☒	The statistical test(s) used AND whether they are one- or two-sided *Only common tests should be described solely by name; describe more complex techniques in the Methods section.*
☒	☐	A description of all covariates tested
☒	☐	A description of any assumptions or corrections, such as tests of normality and adjustment for multiple comparisons
☐	☒	A full description of the statistical parameters including central tendency (e.g. means) or other basic estimates (e.g. regression coefficient) AND variation (e.g. standard deviation) or associated estimates of uncertainty (e.g. confidence intervals)
☐	☒	For null hypothesis testing, the test statistic (e.g. *F*, *t*, *r*) with confidence intervals, effect sizes, degrees of freedom and *P* value noted *Give P values as exact values whenever suitable.*
☒	☐	For Bayesian analysis, information on the choice of priors and Markov chain Monte Carlo settings
☒	☐	For hierarchical and complex designs, identification of the appropriate level for tests and full reporting of outcomes
☐	☒	Estimates of effect sizes (e.g. Cohen's *d*, Pearson's *r*), indicating how they were calculated

Our web collection on statistics for biologists contains articles on many of the points above.

Software and code

Policy information about availability of computer code

Data collection	Proteome Discoverer v1.4, EnSpire Multimode Plate Reader Software, Bio-Rad CFX Manager Software, BD FACSDiva software
Data analysis	Microsoft Excel, GraphPad Prism v7.0, MasterPlex ReaderFit v2.0, ImageJ v1.52c, MaxQuant v1.5.8.3, metaX, InForm Analysis Software, FlowJo

For manuscripts utilizing custom algorithms or software that are central to the research but not yet described in published literature, software must be made available to editors/reviewers. We strongly encourage code deposition in a community repository (e.g. GitHub). See the Nature Research guidelines for submitting code & software for further information.

Data

Policy information about availability of data

All manuscripts must include a data availability statement. This statement should provide the following information, where applicable:

- Accession codes, unique identifiers, or web links for publicly available datasets
- A list of figures that have associated raw data
- A description of any restrictions on data availability

The breast cancer RNA-seq data used to analyze LINK-A expression were derived from the TCGA Research Network: http://cancergenome.nih.gov/, and the breast cancer RNA-seq BAM files were downloaded from the UCSC Cancer Genomics Hub (CGHub, https://cghub.ucsc.edu/). Source data for all human tissue experiments have been provided as Supplementary Table 1. Supplementary Tables 5,6 provide information of oligonucleotides and antibodies used in this study, respectively. The raw RNA-seq data for this manuscript are available at GEO under the accession number (GSE113143). Whole exome sequencing data was deposited to NCBI Sequence Read Archive, with ID as (PRJNA453620). All other data are available from the corresponding author on reasonable request.

Field-specific reporting

Please select the one below that is the best fit for your research. If you are not sure, read the appropriate sections before making your selection.

☒ Life sciences ☐ Behavioural & social sciences ☐ Ecological, evolutionary & environmental sciences

For a reference copy of the document with all sections, see nature.com/documents/nr-reporting-summary-flat.pdf

Life sciences study design

All studies must disclose on these points even when the disclosure is negative.

Sample size	No statistical method was used to predetermine sample size. Sample sizes were determined based on previous studies in the field to enable statistical analyses and ensure reproducibility.
Data exclusions	No data were excluded from this study.
Replication	Each of these experiments was independently repeated for 3-5 times. H & E staining, immunohistochemistry staining or immunoflurosence staining were representative of 3-7 animals.
Randomization	Experiments described here were not randomized.
Blinding	The investigators were not blinded to allocation during experiments and outcome assessment, because all data were acquired from cell or tissue samples of specific genotypes or with designated genetic manipulations.

Reporting for specific materials, systems and methods

We require information from authors about some types of materials, experimental systems and methods used in many studies. Here, indicate whether each material, system or method listed is relevant to your study. If you are not sure if a list item applies to your research, read the appropriate section before selecting a response.

Materials & experimental systems

n/a	Involved in the study
☐	☒ Antibodies
☐	☒ Eukaryotic cell lines
☒	☐ Palaeontology
☐	☒ Animals and other organisms
☒	☐ Human research participants
☒	☐ Clinical data

Methods

n/a	Involved in the study
☒	☐ ChIP-seq
☐	☒ Flow cytometry
☒	☐ MRI-based neuroimaging

Antibodies

Antibodies used

Antibodies Company Catalogue Number Clone Dilution Application
ERalpha Invitrogen MA5-13191 1D5 1:100 (IHC), 1:1000 (IB) IHC, IB
PR Cell Signaling Technology 3153 C89F7 1:100 (IHC), 1:1000 (IB) IHC, IB
HER2 Invitrogen MA5-13675 3B5 1:100 (IHC), 1:1000 (IB) IHC, IB
TAP1 LSBio LS-B14012 Polyclonal 1:200 IHC
TAP2 LSBio LS-B14864 Polyclonal 1:200 IHC
TAPBP/Tapasin LSBio LS-C331792 Polyclonal 1:250 IHC
CALR / Calreticulin LSBio LS-B9387 Polyclonal 1:100 IHC
CD8α Cell Signaling Technology 70306 C8/144B 1:250 IHC
CD8α Cell Signaling Technology 98941 D4W2Z 1:200 IHC, IF
CD3ε Cell Signaling Technology 85061 D7A6E 1:250 IHC, IF
PD-L1 Cell Signaling Technology 13684 E1L3N 1:250 (IHC, IF), 1:1000 (IB) IHC, IF, IB
EpCAM Cell Signaling Technology 93790 E6V8Y 1:250 IHC
PD-L1 Cell Signaling Technology 64988 D5V3B 1:200 IHC
CD3 Novus Biologicals NB600-1441 SP7 1:200 IHC
CTLA-4 Santa Cruz Biotechnology sc-376016 F-8 1:100 IHC
Granzyme B Abcam ab4059 Polyclonal 1:200 IF
p53 Invitrogen MA5-12453 PAb 122 1:50 IHC
Rb Invitrogen MA5-11387 1F8 (Rb1) 1:50 IHC
PCNA Cell Signaling Technology 13110 D3H8P 1:200 IHC
Cleaved Caspase-3 Cell Signaling Technology 9579 D3E9 1:250 IHC
Phospho-PKA C (Thr197) Cell Signaling Technology 5661 D45D3 1:1000 IB
Phospho-TRIM71 (Ser3) Yenzym Antibodies Custom generated Polyclonal 1:1000 IB

TRIM71 Abcam ab105330 Polyclonal 1:1000 IB
TAP1 Santa Cruz Biotechnology sc-376798 B-8 0.180555556 IF
TAP1 Cell Signaling Technology 12341 Polyclonal 1:1000 (IB), 1:500 (IP) IB, IP
Ub-TAP1 (Lys537) Yenzym Antibodies Custom generated Polyclonal 1:1000 IB
TAP2 EMD Millipore MABF945 429.4 1:200 IF
TAP2 Cell Signaling Technology 12259 Polyclonal 1:1000 (IB), 1:500 (IP) IB, IP
Ub-TAP2 (Lys245) Yenzym Antibodies Custom generated Polyclonal 1:1000 IB
TAPBPL Invitrogen MA5-26155 OTI2A4 1:200 IF
TPSN Abcam ab13518 Polyclonal 1:2000 (IB), 1:500 (IP) IB, IP
Ub-TPSN (Lys213) Yenzym Antibodies Custom generated Polyclonal 1:1000 IB
Calregulin Santa Cruz Biotechnology sc-166837 A-9 1:200 IF
CALR / Calreticulin Invitrogen PA3-900 Polyclonal 1:1000 (IB), 1:500 (IP) IB, IP
Ub-CALR (Lys48) Yenzym Antibodies Custom generated Polyclonal 1:1000 IB
HSP90B1 Novus Biologicals NBP2-44690 SPM249 1:200 IF
PD-1 Cell Signaling Technology 86163 D4W2J 1:1000 IB
β2-microglobulin Cell Signaling Technology 12851 D8P1H 1:1000 IB
MHC class I Santa Cruz Biotechnology sc-32235 W6/32 1:1000 IB
Ubiquitin LifeSensors AB120 FK2 1:1000 IB
GAPDH Santa Cruz Biotechnology sc-32233 6C5 1:3000 IB
GST-Tag Cell Signaling Technology 2624 26H1 1:2000 IB
His-Tag Cell Signaling Technology 12698 D3I1O 1:2000 IB
Phosphatidylinositol 3,4,5-trisphosphate Invitrogen A-21328 RC6F8 1:1000 Dot-blot
Gα i-1/2/3 Santa Cruz Biotechnology sc-136478 35 1:2000 IB
CNR2 Abcam ab3561 Polyclonal 1:1000 IB
GABR1 Invitrogen PA5-27725 Polyclonal 1:1000 IB
ADA2A GeneTex GTX55173 Polyclonal 1:1000 IB
ACM4 EMD Millipore MAB1576 17F10.2 1:1000 IB
OPRM1 Invitrogen 44-308G Polyclonal 1:1000 IB
PKA C-α Cell Signaling Technology 4782 Polyclonal 1:1000 IB
FLAG-TAG Sigma-Aldrich F1804 M2 1:3000 IB
K48-Polyubiquitin Cell Signaling Technology 4289 Polyclonal 1:1000 IB
UBE2S Cell Signaling Technology 11878 D5H9H 1:1000 IB
CD45 BioLegend 103106 30-F11 1:100 FC
CD3 BioLegend 100312 145-2C11 1:100 FC
CD8 BioLegend 100714 53-6.7 1:100 FC
H-2Kb BioLegend 116518 AF6-88.5 1:100 FC
H-2Kb bound to SINFEKL BioLegend 141604 25-D1.16 1:100 FC
β 2-microglobulin BioLegend 154504 A16041A 1:100 FC
PD-L1 BioLegend 124312 10F.9G2 1:100 FC
F4/80 BioLegend 123108 BM8 1:100 FC
CD11b BioLegend 101208 M1/70 1:100 FC
Ly6G/Ly6C BioLegend 108412 RB6-8C5 1:100 FC
β 2-microglobulin BioLegend 316304 2M2 1:100 FC
HLA-A, -B, -C BioLegend 311410 W6/32 1:100 FC

Validation

Commercially available antibodies have been validated by various methods as indicated on manufacturers' website. The Ub-TAP1 (Lys537), Ub-TAP2 (Lys245), Ub-TPSN (Lys213) and Ub-CALR (Lys48) antibodies were validated by vendor using the following methods: 1) pre-immune vs. test bleed ELISA; 2) antiserum ELISA; 3) pre-purified serum vs. post-purified serum vs. affinity purified antibody ELISA; 4) SDS-PAGE validation of affinity purified antibody. All validation data are available upon request.

Eukaryotic cell lines

Policy information about cell lines

Cell line source(s)

Human TNBC cell lines: MDA-MB-231, MDA-MB-468, BT549, HCC-1187; human HER2-positive breast cancer cell lines: BT474, SK-BR-3; human ER-positive breast cancer cell lines: MCF7, T47D, ZR-75-1; human normal mammary gland epithelial cell line: MCF10A and mouse mammary gland epithelial cell line: NMuMG were purchased from American Type Culture Collection (ATCC); SUM-149 (human TNBC cell line), B16-OVA (mouse melanoma cell line, a gift from H. Patrick), B16F10 (mouse melanoma cell line) were maintained using standard media and conditions.
B16F10 were constructed to stably express LINK-A by selection with G418 (1500μg/ml).
Human target PKAalpha cat, PKAbeta cat, CNR2, ADA2A, GABR1, ACM4 and OPRM specific sgRNA sequences are listed in Supplementary Table 5. MDA-MB-231 and MCF10A cells were constructed to stably express Cas9 and sgRNAs by selection with puromycin (1μgml−1). Single clones were obtained by serial dilution. LINK-A PtdIns(3,4,5)P3-binding motif-deficient cell lines were generated using the CRISPR/Cas9 genome editing system by the Gene Editing/Cellular Model Core Facility (MD Anderson Cancer Center).

Authentication

The cell lines were authenticated by short tandem repeats (STR) profiling performed by MDACC Characterized Cell Line Core Facility.

Mycoplasma contamination

All of the cell lines were free of mycoplasma contamination tested by MDACC Characterized Cell Line Core Facility using MycoAlert kit.

Commonly misidentified lines (See ICLAC register)	No commonly misidentified cell lines were used.

Animals and other organisms

Policy information about studies involving animals; ARRIVE guidelines recommended for reporting animal research

Laboratory animals	For experiments involved LINK-A knock-in model 12-weeks old female FVB mice were used. All mice were on a FVB genetic background. We crossed Tg-LINK-A mice with MMTV-cre mice [Tg(MMTV-cre)1Mam, The Jackson Laboratory] to produce mice with LINK-A transgene expression in the mammary glands. For establishment of syngeneic MMTV-Tg(LINK-A) model, MMTV-Tg(LINK-A) mice bearing mammary tumors up to 600 mm3 were euthanized, and the tumor was excised. Tumors were dissociated as a single cell using the gentle MACS Dissociator (Miltenui Biotec Inc) with the mouse Tumor Dissociation kit (Miltenui Biotec). A single-cell suspension was generated after filtration through a 70-mm cell strainer (BD Falcon). Single-cell suspensions from freshly harvested MMTV-Tg(LINK-A) mammary tumors were counted and resuspended in transplantation buffer containing 50% growth factor–reduced Matrigel (BD Pharmingen). Cells (40,000 per gland) were injected into the right inguinal fat pad of 4-week-old female FVB/N recipients.
Wild animals	This study does not include wild animals.
Field-collected samples	This study does not include field-collected samples.
Ethics oversight	Institutional Animal Care and Use Committee (IACUC) of the University of Texas M.D. Anderson Cancer Center (MDACC)

Note that full information on the approval of the study protocol must also be provided in the manuscript.

Flow Cytometry

Plots

Confirm that:

☒ The axis labels state the marker and fluorochrome used (e.g. CD4-FITC).

☒ The axis scales are clearly visible. Include numbers along axes only for bottom left plot of group (a 'group' is an analysis of identical markers).

☒ All plots are contour plots with outliers or pseudocolor plots.

☒ A numerical value for number of cells or percentage (with statistics) is provided.

Methodology

Sample preparation	Cell lines: 1 × 106 cells per condition were stained with the appropriate antibodies diluted in DPBS (Corning) plus 2% FBS (Gibco) for 30 min at 25°C. Matched fluorescence minus one (FMO) staining for each condition was performed as a control. Mouse tissues and tumors: mouse tissues and tumors were dissociated as a single cell using the gentleMACS Dissociator (Miltenui Biotec Inc) with the mouse Tumor Dissociation kit (Miltenui Biotec), after lysis of red blood cells (RBC Lysis Buffer, BioLegend), single-cell suspensions were blocked with anti-CD16/32 (BioLegend) for 20 min on ice and then incubated with appropriate antibodies for 30 min on 25°C. Mouse antibodies: antibodies were purchased from BioLegend unless otherwise indicated: CD45, CD3, CD8, H-2Kb, H-2Kb bound to SINFEKL, beta2-microglobulin, PD-L1, F4/80, CD11b, Ly6G/Ly6C. Human antibodies: beta2-microglobulin and HLA-A, -B, -C. To distinguish live/dead cells, Zombie Violet (BioLegend) fixable viability dyes were used.
Instrument	BD LSR II flow cytometer
Software	BD FACSDiva software and FlowJo
Cell population abundance	The single cell suspension from mouse tumor tissues were staining with APC-conjugated anti-CD3, APC-Cy7-conjuated anti-CD8 and PE-conjugated anti-CD45, then CD3+CD8+CD45.1+ cells were sorted in BD LSR II with purity of priority.
Gating strategy	A gate was set on the live cell population based on the forward and side scatter properties, and the gating strategy has been provided in Supplementary Fig.8i

☒ Tick this box to confirm that a figure exemplifying the gating strategy is provided in the Supplementary Information.